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Identification and Characterization of Insulin-Like Growth Factor Receptors on Adult Rat Cardiac Myocytes: Linkage to Inositol 1,4,5-Trisphosphate Formation*

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ABSTRACT. Cultured cardiac myocytes from adult Sprague-Dawley rats express both insulin-like growth factor-I (IGF-I) receptors and insulin-like growth factor-II/mannose 6-phosphate (IGF-II/Man6P) receptors and respond to IGF-I with a dose-dependent accumulation of inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] and inositol 1,4-bisphosphate [Ins(1,4)P_2]. Specific binding of [^{125}I]IGF-I to isolated membranes from cultured cardiac myocytes amounted to 1–1.2%. Binding of [^{125}I]IGF-I was inhibited by unlabeled IGF-I at nanomolar concentrations and insulin at much higher concentrations. These data suggest that IGF-I binds to its own receptor on rat cardiac myocytes. Competitive binding studies using isolated membranes from cardiac myocytes and [^{125}I]IGF-II showed 2–4% specific binding. Binding of [^{125}I]IGF-II was inhibited by IGF-II and much less potently by IGF-I and insulin. Immunoglobulin G (IgG) 3637 (an IgG directed against the IGF-II/Man6P receptor) partially inhibited binding of [^{125}I]IGF-II whereas nonimmune IgG did not. Affinity cross-linking studies with [^{125}I]IGF-II and cardiac myocyte membranes and subsequent analysis of the ligand-receptor complex using SDS-PAGE and autoradiography showed a radiolabeled band of approximately 250 kilodalton (kDa). The formation of the [^{125}I]IGF-II-receptor complex was inhibited by incubation with IGF-II and IgG 3637 but not by insulin or nonimmune IgG.

Western blotting of protein extracts from cultured cardiac myocytes was performed using IgG 3637 and an immunoperoxidase technique for the visualization of the IGF-II/Man6P receptor protein. A specific band at 220 kDa under nonreducing conditions was detected on the blots, providing further evidence for the expression of the IGF-II/Man6P receptor by cardiac myocytes.

The effect of IGFs on the accumulation of inositol phosphates was measured by HPLC analysis of perchloric acid extracts from myo-[^{3}H]inositol-labeled cultured cardiac myocytes. IGF-I (50 ng/ml) stimulated the accumulation both of Ins(1,4,5)P_3 and Ins(1,4)P_2 after 30 sec by 43% and 63%. IGF-II (up to 500 ng/ml) had no significant effect on inositol phosphate accumulation under the same conditions. However, in the presence of millimolar concentrations of Man6P, IGF-II (500 ng/ml) also increased Ins(1,4,5)P_3 accumulation by 59%. We conclude that cardiac myocytes from adult rats express IGF receptors and respond to IGFs with the accumulation of Ins(1,4,5)P_3 and Ins(1,4)P_2. This effect seems to be mediated by an IGF-I receptor-specific pathway. (*Endocrinology* **130:** 145–151, 1992)

THE insulin-like growth factors (IGF-I and IGF-II) are polypeptide hormones closely related to insulin in regard to their amino acid sequence and biological activity (1, 2). IGF-I mediates the anabolic action of GH *in vivo*. The *in vivo* role of IGF-II is less clear. The IGFs bind to high affinity receptors present on many cells and in many tissues (3). The IGF-I receptor is a heterotetramer, made of two α-binding subunits [(130 kilodaltons kDa)] and two β-subunits (95 kDa) which contain tyrosine kinase activity. The IGF-I receptor is highly homologous to the insulin receptor and is thought to mediate the biological effect of both IGF-I and IGF-II (3, 4). The primary structure of the IGF-II receptor was shown by complementary DNA cloning to be identical to the structure of the cation-independent mannose 6-phosphate receptor (Man6P) (5–9). It is clear that this receptor targets acid hydrolases to lysosomes and also serves as a degradative pathway for IGF-II (10–13). It has also been suggested that the IGF-II/Man6P receptor functions as a signaling receptor for IGF-II mediated biological actions such as stimulation of DNA-synthesis and cell

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proliferation in BALB/c 3T3 cells (14, 15), clonal growth of K562 erythroleukemia cells (16), Na^+/H^+-exchange in proximal tubular cells from canine kidney (17), stimulation of glycogen synthesis in a human hepatoma cell line (Hep 62) (18). In addition, IGF-II has also been implicated in modulating lysosomal enzyme trafficking via the IGF-II/Man6P receptor (19, 20).

IGF receptors have been found in heart tissue (21, 22) and a positive inotropic effect of insulin and both IGF-I and IGF-II on isolated fetal rat cardiac myocytes has been described (23).

For many other agonists which elicit positive inotropic responses specific receptors have been found in mammalian heart. In addition, the release of inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] as an initial event in receptor-mediated activation of myocardial phosphoinositidase C has been described for a number of such receptors, for example the α_1-adrenoceptor (24), the purinoceptor P_2 (25), and the histamine H_1 receptor (26).

Although the physiological responses to IGFs are well described in several cell types, the signal transduction mechanisms by which IGF-I and IGF-II transmit their cellular signal remain to be elucidated (27).

Contradictory data about the effects of IGFs on inositol trisphosphate generation (28–30) prompted us to investigate 1) the presence and binding characteristics of the IGF-I- and the IGF-II/Man6P-receptor and, 2) the effects of IGF-I and IGF-II on inositol polyphosphate accumulation in cultured cardiac myocytes.

Rat cardiac myocytes were chosen as a model since 1) $InsP_3$ accumulation can be studied directly in a defined primary culture system of a homogenous cell population, and 2) such cells have been reported to respond to IGFs (23).

Materials and Methods

Cell culture, myo-[^{3}H]inositol labeling and stimulation experiments

The procedure of isolating cardiac myocytes from adult Sprague-Dawley rats (10–14 weeks old, obtained from the Zentralinstitut für Versuchstierzucht, Hannover, Germany) was carried out as described recently (31). The use of the animals was approved by the Freie und Hansestadt Hamburg, Gesundheitsbehörde. In brief, hearts were removed and perfused in a recirculating manner for 30 min with 60 ml Krebs-Ringer-bicarbonate buffer containing 5500 U collagenase (type II, Biochrom, Berlin, Germany). Then atria were removed and the ventricles were cut in 20 ml Krebs-Ringer-bicarbonate buffer containing 2750 U collagenase. Rod-shaped cardiomyocytes were obtained by two centrifugation steps (20 × *g*, 60 sec). The Ca^{++}-concentration was raised in 10 steps up to 0.5 mM, followed by another centrifugation step (20 × *g*, 60 sec). The myocytes were then incubated for 2 h in medium 199 (Biochrom) containing fetal calf serum (4%), gentamycin, and 1.8 mM Ca^{++}. Nonadherent cells were removed and the rod-shaped myocytes were labeled for 6 h in serum-free medium 199 containing myo-[^{3}H]inositol (20 μCi/ml, SA, 80–120 Ci/mmol). After labeling, the myocytes were incubated in Krebs-Ringer-HEPES buffer containing 10 mM LiCl (and 10 μM propranolol when (−)-noradrenaline was used for stimulation) and then exposed to IGFs, (−)-noradrenaline, or carbamoylcholine for 30 sec. The incubation was terminated by addition of $HClO_4$ (10%, vol/vol). Samples were transferred to Eppendorf tubes, precipitated protein was removed by centrifugation (10,000 × *g*, 10 min), and the supernatants were titrated to pH 4 to 5. $KClO_4$ was removed by centrifugation (10,000 × *g*, 10 min) and the supernatants were subjected to HPLC analysis.

HPLC analysis of inositol phosphates

Inositol phosphates were separated by anion exchange-HPLC using a Merck-Hitachi LC-5000 liquid controller and a L-6000 pump equipped with a Partisil 10 SAX column (Whatman, Clifton, NJ) by a method originally introduced by Dean and Moyer (32). The method was modified to elute $InsP_4$ from the column as described recently (24). Quantitation of inositol phosphate accumulation was carried out by liquid scintillation counting (Packard TriCarb 460, Frankfurt, Germany) of 1 ml or 0.5 ml fractions mixed with 5 ml or 2.5 ml Ready Value scintillation cocktail (Beckman, München, Germany) for 9 min or 30 min. These long counting times were necessary to obtain reliable data from samples with low radioactivity.

Preparation of cardiomyocyte membranes

Cardiac myocytes from one rat heart were homogenized in 2 ml membrane isolation buffer (10 mM HEPES, 230 mM mannitol, 70 mM sucrose, 50 ng/ml phenylmethylsulfonylfluoride, 50 ng/ml antipain, pH 7.0) by 10 strokes of a glass/teflon-homogenizer. Crude membranes were prepared by two centrifugation steps: removal of cell fragments and nuclei at 1000 × *g* (10 min, 4 C) and separation of the cytosolic fraction from the total membrane fraction at 100,000 × *g* (60 min, 4 C, Kontron centrifuge T-2060).

Solubilization of IGF-II/Man6P-receptors

Cultured myocytes were treated with Tris buffer containing 20 mM Tris-HCl, pH 7.4, 2% (vol/vol) Triton X-100, 50 ng/ml phenylmethylsulfonylfluoride, 50 ng/ml antipain (about 1 ml/2.4×10^5 cells) for 30 min at 4 C. The lysate was harvested with a rubber policeman and transferred to Eppendorf tubes and stored frozen at −20 C.

Polyacrylamide gel electrophoresis (PAGE)

Sodium dodecyl sulfate (SDS)-PAGE was performed using the discontinuous buffer system of Laemmli (6% acrylamide-bis) (33).

Antireceptor antibody

Antiserum 3637 has been raised against IGF-II receptor that had been purified from Swarm rat chondrosarcoma cells by affinity chromatography on IGF-II sepharose (13). The antibody is specific for rat IGF-II/Man6P receptor and does neither recognize the IGF-I or insulin receptor nor the IGF binding proteins (13, 34). Also, antibody 3637 does not recognize the cation-dependent 46 kDa Man6P receptor (35).

Western blotting of IGF-II/Man6P receptors

The Western blotting procedure was essentially performed as described previously (13). In brief, cardiac myocytes were solubilized and subjected to SDS-PAGE (6% gel) under non-reducing conditions. The proteins were transferred onto nitrocellulose paper by electroelution and the nitrocellulose sheets incubated with antiserum 3637. Antirabbit-immunoglobulin G (IgG) antiserum and a biotin-avidin horse radish peroxidase system (Vectastain) were used to detect receptor-antibody complexes on the nitrocellulose sheets.

Binding of [^{125}I]IGF-I and [^{125}I]IGF-II to cardiomyocyte membranes

Binding of [^{125}I]IGF-I and [^{125}I]IGF-II to cardiomyocyte membranes (0.85 mg membrane protein/400 μl) was carried out in 1.5 ml microfuge tubes for 2 h at room temperature in Dulbecco's modified Eagle's medium (DMEM) with 0.2% BSA with labeled and unlabeled peptides or IgGs present as indicated. Bound from free radioactivity was separated by centrifugation in a Hettich microfuge.

Affinity cross-linking of [^{125}I]IGF-II to cardiomyocyte membranes

Affinity cross-linking was carried out using disuccinimidyl suberate (DSS) as the cross-linking reagent (Pierce Chemicals, Rockford, IL). Crude membranes from rat cardiac myocytes were incubated with radioligands, unlabeled polypeptides, and IgGs as indicated for 2 h at room temperature in DMEM with 1% BSA. The membranes were centrifuged, washed once with cold PBS, pH 7.4, and resuspended in DMEM with 0.1 mM DSS without BSA. After 10 min at room temperature, the reaction was quenched with 10 mM Tris, 1 mM EDTA solution for 5 min at room temperature, the samples were centrifuged in a microfuge for 3 min, and the liquid was discarded. Sample buffer was added, and the samples were boiled for 3 min and analyzed by SDS-PAGE (0.1% SDS, 6% acrylamide) and subsequent autoradiography (Kodak X-Omat film).

Materials

Recombinant human IGF-I and IGF-II were a kind gift from Dr. K. Müller, Ciba Geigy (Basel, Switzerland) or donated by Eli Lilly (Indianapolis, IN). Rat IGF-II (MSA-III-2) was kindly given by Dr. S. P. Nissley, National Institutes of Health (Bethesda, MD). Insulin and mannose 6-phosphate were purchased from Sigma Biochemicals (Taufkirchen, Germany). IGF-II was radioiodinated by a modified chloramine-T procedure to specific activities of 50–100 Ci/g (36). [^{125}I]IGF-I (SA, 2000 Ci/mmol) was purchased from Amersham Buchler (Braunschweig, Germany).

All other materials were obtained as described in an earlier paper (31).

Results

Rat cardiac myocytes express high affinity binding sites for [^{125}I]IGF-I and [^{125}I]IGF-II

Competitive binding studies using crude membranes from isolated rat cardiac myocytes and [^{125}I]IGF-I showed 1–1.2% specific binding. Half-maximal displacement of [^{125}I]IGF-I binding occurred at approximately 1.5 ng/ml at a membrane protein concentration of 2.125 mg/ml. Insulin at high concentrations (5–25 μg/ml) also completely blocked binding of [^{125}I]IGF-I to rat cardiac myocyte membranes. This result strongly suggests binding of [^{125}I]IGF-I to the IGF-I receptor present on cardiac myocytes of adult rat hearts (Fig. 1A).

Competitive binding studies using crude membranes from isolated cardiac myocytes and [^{125}I]IGF-II showed 2–4% specific binding. Half-maximal displacement of [^{125}I]IGF-II binding occurred at 30 ng/ml IGF-II at a membrane protein concentration of 2.125 mg/ml (Fig. 1B). IGF-I was much less potent, anti IGF-II/Man6P receptor IgG competed partially, whereas nonimmune rabbit IgG was less effective (data not shown). Interestingly, insulin at high concentrations (5–25 μg/ml) also displaced [^{125}I]IGF-II binding partially (data not shown). These results suggest that [^{125}I]IGF-II under the conditions of these experiments might bind both to the IGF-II/Man6P receptor (IgG 3637 competable site) and the IGF-I receptor (insulin competable site).

Affinity cross-linking experiments were performed using cardiomyocyte membranes, [^{125}I]IGF-II and DSS suberate as the cross-linking agent. Analysis of ligand-receptor complexes by SDS-PAGE and autoradiography was carried out as described in *Materials and Methods*.

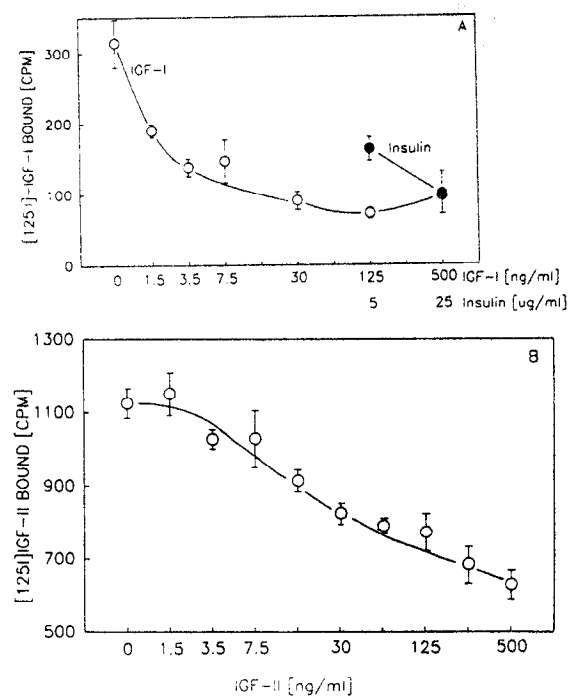

FIG. 1. Binding of [^{125}I]IGF-I and [^{125}I]IGF-II to crude membranes from isolated cardiac myocytes. A, [^{125}I]IGF-I was incubated with cardiac myocyte membranes (0.85 mg membrane protein/400 μl) in the presence of various concentrations of unlabeled IGF-I or insulin for 2.5 h as described in *Materials and Methods*. B, [^{125}I]IGF-II was incubated with cardiac myocyte membranes (0.85 mg membrane protein/400 μl) in the presence of various concentrations of unlabeled IGF-II for 2.5 h as described in *Materials and Methods*. Data are mean values ± SD from three independent experiments.

A specific band with an approximate mol wt of 250 k under reducing conditions was detected. IGF-II (2 μg/ml) and IgG 3637 (180 μg/ml) completely abolished the formation of this band whereas nonimmune IgG and insulin did not (Fig. 2A). We conclude from these data that cardiac myocytes express IGF-II/Man6P receptors. Further evidence for the presence of IGF-II/Man6P receptors on cardiac myocytes is derived from Western blotting of the IGF-II/Man6P receptor in protein extracts from cultured cardiac myocytes. A specific band with an apparent mol mass of 220 k under nonreducing conditions was detected in all protein extracts [seven different protein preparations from three independent cardiac myocyte preparations (Fig. 2B)]. The protein bands at about 72 to 80 kDa were also seen when nonimmune rabbit serum was used instead of antiserum 3637, indicating a nonspecific interaction with the detection system rather than receptor degradation products.

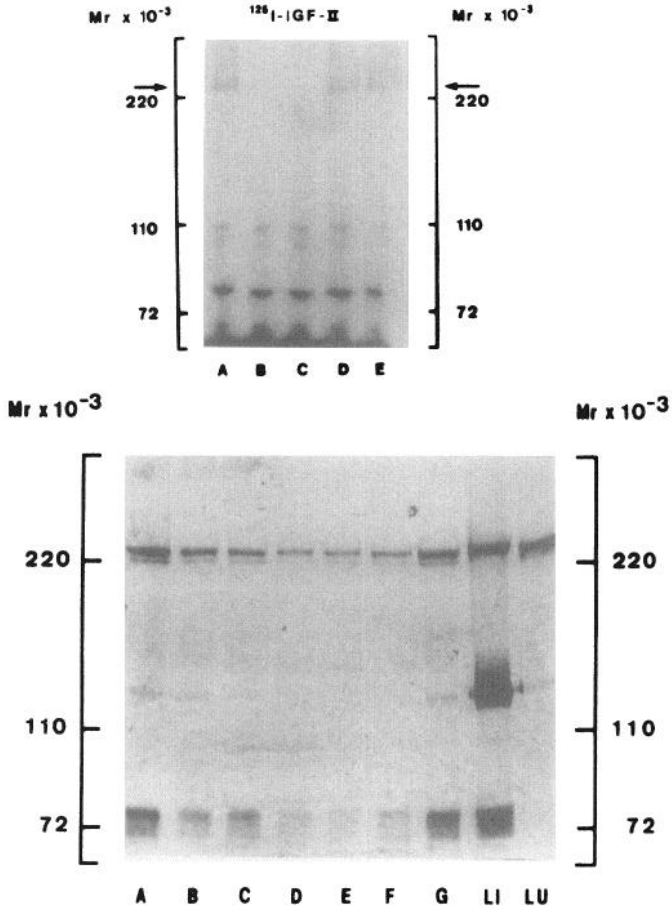

FIG. 2. *Top*, Affinity cross-linking of [^{125}I]IGF-II to rat cardiomyocyte membranes. [^{125}I]IGF-II was incubated with crude membranes from isolated cardiac myocytes, and DSS was used as the cross-linking reagent as described in *Materials and Methods* in the absence (lane A) or presence (lane B) of 2 μg/ml IGF-II, and in the presence of 180 μg/ml IgG 3637 (lane C), 180 μg/ml nonimmune IgG (lane D), or 25 μg/ml insulin (lane E). *Bottom*, Western blotting of the solubilized IGF-II/Man6P receptor from cultured rat cardiac myocytes. Western blotting of IGF-II/Man6P receptors was performed as described in *Materials and Methods*. In brief, solubilized extracts from cultured cardiac myocytes (lanes A–G) and extracts from rat liver (lane LI) and rat lung (lane LU) were subjected to SDS-PAGE under nonreducing conditions. Proteins were then transferred onto nitrocellulose paper and incubated with antiserum 3637. Antirabbit-IgG antiserum and a biotin-avidin horse raddish peroxidase system were used to detect receptor-antibody complexes. Protein bands of lower molecular weights were also seen when nonimmune rabbit serum was used instead of antiserum 3637 and therefore represent nonspecifically stained proteins. One representative blot out of three independent experiments is shown.

IGFs stimulate inositol polyphosphate accumulation in cardiac myocytes

Inositol phosphates were measured after stimulation of cardiac myocytes with IGFs, (−)-noradrenaline, carbamoylcholine, or IGF-II in combination with Man6P as described in *Materials and Methods*. Stimulation of cultured cardiac myocytes with nanomolar concentrations of IGF-I for 30 sec resulted in a significant increase both in $Ins(1,4,5)P_3$ and inositol 1,4-bisphosphate [$Ins(1,4)P_2$] indicating rapid activation of phosphoinositidase C as well as rapid degradation of $Ins(1,4,5)P_3$ by 5-phosphomonoesterase activity (Fig. 3). Other inositol phosphate compounds, in particular intermediates of the inositol tetrakisphosphate pathway such as $Ins(1,3,4,5)P_4$, $Ins(1,3,4)P_3$, $Ins(1,3)P_2$, and $Ins(3,4)P_2$ were not affected by IGF-I during the early stimulation period (data not shown).

IGF-II was used for stimulation alone or in combination with Man6P as it was recently reported (29) that Man6P enhances the IGF-II-mediated activation of phosphoinositidase C. Even high concentrations of IGF-II (500 ng/ml) had no significant stimulatory effect on inositol phosphate accumulation after 30 sec (Fig. 3). Interestingly, when IGF-II (250 ng/ml or 500 ng/ml) was added in the presence of Man6P (5 mM), a significant increase of $Ins(1,4,5)P_3$ was observed (Fig. 3). Neither the direct phosphorylation or dephosphorylation products of $Ins(1,4,5)P_3$, $InsP_4$, and $Ins(1,4)P_2$ (Fig. 3), nor any other known inositol phosphates (data not shown) were found to be elevated within 30 sec of IGF-II or combined IGF-II/Man6P-treatment of cardiac myocytes. Man6P at millimolar concentrations had no effect on $Ins(1,4,5)P_3$ accumulation when tested alone.

Stimulation of inositol phosphate accumulation by pharmacological agents via α_1-adrenergic and muscarinic cholinergic receptors, which are coupled to cardiac phosphoinositidase C, induced a more pronounced effect on inositol polyphosphate accumulation than IGF-I or IGF-II/Man6P (Table 1). In particular α_1-adrenergic stimulation by 50 μM (−)-noradrenaline (in the presence of 10 μM propranolol) provoked an approximately 10-fold higher $Ins(1,4,5)P_3$ release when compared with the stimulatory effect of nanomolar concentrations of IGF-I.

Discussion

It has been suggested in the literature that IGF receptors are present in rat heart (21, 22). We demonstrate

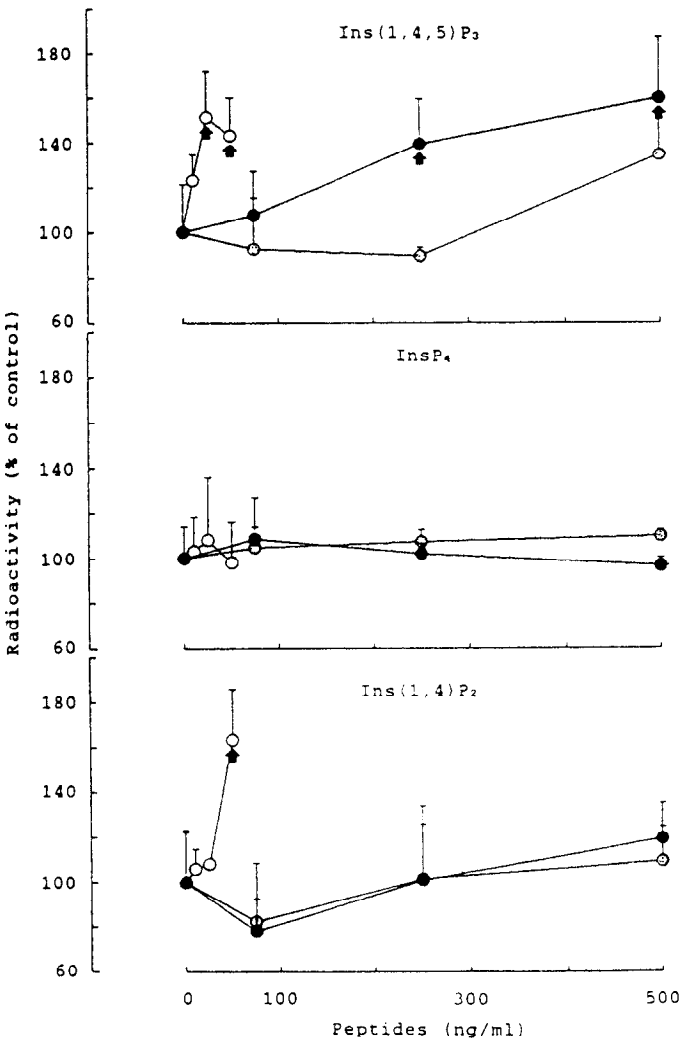

FIG. 3. Effect of IGFs on inositol polyphosphate accumulation in rat cardiac myocytes. Cardiac myocytes were cultured and prelabeled with myo-[^{3}H]inositol as described in *Materials and Methods*. Stimulation of cells with IGF-I (○), IGF-II (○), or IGF-II in combination with Man6P (5 mM; ●) was carried out in KRH-buffer at 38 C for 30 sec. Neutralized perchloric acid extracts were analyzed for inositol phosphates by HPLC. Values are presented as mean ± SD. (n = 3–5). Radioactivity of inositol phosphates in samples from unstimulated cells was (in desentegrations per min/10^6 cells): Ins(1,4,5)P_3 180, $InsP_4$ 24, Ins(1,4)P_2 420. Usually, 3–3.5 × 10^5 cells were plated on one culture dish and extracts from three to four dishes were pooled for analysis. The liquid counting efficiency ranged between 30% and 40% depending on the salt content of the samples. For an accurate quantification of radioactivity long counting periods were chosen, *e.g.* 3 × 3 min or 3 × 10 min/sample. *Arrows* indicate significant differences from control values according to analysis of variance (multifactor analysis of variance, $P \leq 0.05$).

here that both IGF-I receptors and IGF-II/Man6P receptors in rat heart are actually expressed by cardiac myocytes.

Sklar *et al.* (21) using total protein lysates from rat hearts and immunoblotting demonstrated the expression of IGF-II/Man6P receptors in rat hearts at different ages. Engelmann *et al.* (22) used total membrane preparations from adult rat hearts and binding and affinity

TABLE 1. Effect of various agonists on inositol phosphate accumulation in rat cardiac myocytes

	Ins(1,4,5)P_3	$InsP_4$	Ins(1,4)P_2
	(fold stimulation of control)		
Control	1.00 ± 0.18	1.00 ± 0.50	1.00 ± 0.17
IGF-I (50 ng/ml)	1.48 ± 0.21[a]	1.08 ± 0.28	1.64 ± 0.36[a]
IGF-II (500 ng/ml)	1.34 ± 0.20	1.10 ± 0.03	1.09 ± 0.26
IGF-II/Man6P (500 ng/ml, 5 mM)	1.59 ± 0.27[a]	0.97 ± 0.04	1.19 ± 0.52
Noradrenaline (50 μM) (+ propranolol 10 μM)	6.19 ± 1.19[b]	10.06 ± 0.97[b]	7.10 ± 1.32[b]
Carbachol (100 μM)	1.99 ± 0.42[b]	4.92 ± 1.74[b]	1.78 ± 0.25[b]

Cardiac myocytes were cultured and prelabeled with myo-[^{3}H]inositol as described in *Materials and Methods*. Stimulation was carried out in KRH-buffer at 38 C for 30 sec. Neutralized perchloric acid extracts were analyzed for inositol phosphates by HPLC. Values are presented as mean ± SD (n = 4–9). Radioactivity of inositol phosphates in samples from unstimulated cells was (in disintegration per min/10^6 cells): Ins(1,4,5)P_3 180, $InsP_4$ 24, Ins(1,4)P_2 420.

[a] Significant differences to control values according to analysis of variance (multifactor analysis of variance, $P \leq 0.05$).

[b] $P \leq 0.01$.

cross-linking studies to conclude that IGF-I receptors are present in rat heart. The cultured cardiac myocytes used in our experiments are homogenous preparations with 5 ± 1.8% (n = 6) of contaminating cells (Gercken, G., and Achterberg, V., unpublished results). Taking into account that the myocyte content of the whole heart is only some 12% of the total number of cells (37), the use of an about 95% pure population means a great improvement compared to conventional models, *e.g.* whole heart preparations or papillary muscles. Using membranes from such cardiac myocytes, we found that both [^{125}I] IGF-I and [^{125}I]IGF-II bind to specific high affinity binding sites with the binding characteristics of the IGF-I and the IGF-II/Man6P receptor respectively. Our affinity cross-linking and immunoblotting data further confirm the presence of the IGF-II/Man6P receptor on adult rat cardiac myocytes. Most importantly our findings indicate not only the presence of the IGF receptors on cardiac myocytes, but also that the IGF-I receptor might actually be coupled to phosphoinositidase C in these highly differentiated cells. Stimulation of cardiac myocytes with nanomolar concentrations of IGF-I led to a significant increase of Ins(1,4,5)P_3 and Ins(1,4)P_2. Other inositol phosphorylation or dephosphorylation products were not affected by stimulation of cells with IGF-I. The finding that the IGF-I receptor is linked to phosphoinositidase C is consistent with a very recent report from Takasu *et al.* (38). These authors found a dose-dependent effect of IGF-I ($EC_{50} \approx 10$ ng/ml) on inositol trisphosphate formation and Ca^{++}-release in cultured porcine thyroid cells. In contrast to the finding of Takasu *et al.* in thyroid cells (38), we could not detect a marked increase in inositol monophosphate ($InsP_1$) after treatment with IGF-I in cardiac myocytes. This discrepancy may be due to the different incubation periods used.

Increased accumulation of $InsP_1$ in cardiac myocytes was observed only after 15 min of muscarinic cholinergic stimulation (31). Therefore it seems unlikely that IGF-I, which exerts a weaker effect than carbamoylcholine in cardiac myocytes, leads to a significant increase of $InsP_1$ after 30 sec. Alternatively, IGF-I might elucidate distinct effects on the accumulation of inositol phosphates in different cell systems.

It has been reported in the literature that IGF-I induces a positive inotropic effect in cardiac myocytes (23). The concentration of IGF-I needed for maximal inotropic effects in the study by Vetter *et al.* (23) are in the same range as the concentrations of IGF-I needed to induce inositol phosphate accumulation in our study. Stimulation of other myocardial receptors such as the α_1-adrenoceptor (24, 39), the histamine receptor H_1 (26), and the purinoceptor P_2 (25) resulted in phosphoinositidase C activation as well as in an increase in force of contraction. Thus, it might well be that IGF-I exerts a positive inotropic effect in cardiac myocytes via a phosphoinositidase C-mediated second messenger pathway. Although the increase of $Ins(1,4,5)P_3$ was markedly lower after IGF- than after α_1-adrenergic- or muscarinic cholinergic stimulation in cardiac myocytes, it should be mentioned, that for these studies (24, 31) pharmacological agents at very high concentrations have been used. Therefore it appears unlikely that physiological concentrations of IGFs would exert the same increases in inositol phosphates. This suggestion is further supported by the finding that stimulation of α_1-adrenoceptors without β-adrenoceptor blockade (an experimental design that mimics a more physiological situation) resulted in a markedly diminished increase in all inositol phosphates in cardiac myocytes (Guse, A. H., I. Berg, and G. Gercken, in press).

IGF-II, in the presence of millimolar concentrations of Man6P seems to mediate its, albeit weak, effect on inositol phosphate formation via the IGF-I receptor as is suggested by the high concentration needed. Many other biological effects of IGF-II are also thought to be mediated via the IGF-I receptor (13, 40). In renal proximal tubular membranes, to our knowledge the only system where IGF-II-induced $Ins(1,4,5)P_3$ formation was observed, a 3- to 4-fold increase of $Ins(1,4,5)P_3$ was measured after stimulation with 10^{-9} M IGF-II (~7 ng/ml), whereas IGF-I even at micromolar concentrations had no effect (28). In cardiac myocytes, we were unable to provoke any effect on inositol phosphate accumulation at such low concentrations of IGF-II (data not shown). Interestingly, in a second report Rogers and Hammerman (29) showed that millimolar concentrations of Man6P enhanced IGF-II-induced $Ins(1,4,5)P_3$-formation in proximal tubular membranes. Man6P also increased the binding of IGF-II to the IGF-II/Man6P receptor in several cell systems (19, 29, 41, 42). However, the stimulatory effect of Man6P is not a universal finding and seems to depend upon the ligand and the system studied (7, 43, 44). It is possible that millimolar concentrations of Man6P may have modulated the amount of IGF-II available for binding to the effective receptor system resulting in the leftwardshift of the dose-response curve for IGF-II in our as well as in Rogers and Hammerman's study (29).

Finally, in another recent study Thrakker *et al.* (30) reported that in isolated human hepatocytes IGF-I, IGF-II, and insulin had no stimulatory effect on inositol phosphate accumulation. This lack of effect of IGFs may potentially be explained by the method used for measuring the inositol phosphate response. Thrakker *et al.* (30) counted an aliquot from the trichloroacetic supernatant of hepatocyte lysates without any separation of inositol from the inositol phosphates. Thus, most of the radioactivity measured in their study might have been [^{3}H]inositol and only a minor part represented [^{3}H] inositol phosphates. Small changes in inositol phosphate accumulation, especially those of $Ins(1,4,5)P_3$ could not have been detected in such a system.

In conclusion, our results indicate not only the presence of IGF receptors on cardiac myocytes, but also evidence the linkage of the IGF-I receptor to the inositol phosphate system.

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